DEVELOPMENTAL REGULATION OF STEROL CARRIER PROTEIN 2 IN CHICKEN INTESTINE AND LIVER AT TIME OF HATCHING[1]

SUMMARY: Sterol Carrier Protein 2, a protein thought to be involved in various aspects of intracellular sterol transport and metabolism, was studied in the small intestine and liver of chickens near hatching. In the intestine, a sudden shift in molecular mass, from 12 kDa to 64 kDa was noted by Western blotting on the day of hatching. Immediately post-hatching, the molecular mass returned to 12 kDa but the protein was several fold more abundant than prior to hatching. In the liver, several molecular mass forms were present at all ages examined but total shift to the 64 kDa form was not seen. Following hatching, however, the 12 kDa form became more abundant. Regardless of age of bird or molecular mass, the protein was found mainly in peroxisomes.

Intracellular lipid transport is important for membrane biogenesis, lipid secretion, storage and conversion to bioactive substances, energy metabolism and the control of lipid biosynthesis (1). One protein thought to be involved in lipid transport is sterol carrier protein 2 (SCP_2), which also is called non-specific lipid transfer protein (nsLTP) (2). This protein has been implicated in modulation of cholesterol synthesis (3,4,5), sterol esterification (6) and conversion to bile acids (7,8) and steroid hormones (9) in mammalian cells. It was first characterized as a 12 kDa species, but it recently has been shown to be present as larger species of approximately 36 and 58 kDa (10,11,12). We demonstrated the presence of SCP_2 in the chicken but have not yet determined its physiological role (13). Its relative abundance in several embryonic chicken tissues which are active in lipid transport or metabolism (yolk sac membrane, liver and small intestine) did not appear to correlate with

the rate of lipid secretion. It did not, for instance, increase in abundance in yolk sac membrane during the final week of embryonic development, when lipid flux and cholesterol esterification rise dramatically compared with earlier periods of development. It was noted, however, that the relative abundance of the different forms of SCP_2 changed dramatically during development. Within a few hours of hatching, the 12 kDa form of SCP_2 nearly disappeared from intestinal tissue, but was replaced by a 64 kDa protein.

Previously we observed that the 12 kDa SCP_2 was the major form of the protein found in adult chicken intestine. Therefore, we wanted to determine at what time the 64 kDa form of SCP_2 was replaced by the low molecular mass form. Here, we report that the shift in molecular mass of chicken intestinal SCP_2 from 12 to 64 kDa and back to 12 kDa tightly coincides with time of hatching. Following hatching, the relative abundance of the 12 kDa form is increased compared to before hatching. While no comparable shift in molecular mass of SCP_2 occurs in liver tissue, the relative abundance of the 12 kDa form of the protein increases after hatching. The tight regulation of SCP_2 abundance and mass in chicken intestine makes this an interesting system in which to examine the molecular biology of SCP_2 synthesis.

MATERIALS AND METHODS

Growth of Embryos and Chicks. Fertile eggs of broiler type chickens were obtained from Moyer's Chicks, Inc., Quakertown, PA. Incubation was in a Lyon Electric Company humidified incubator at 38°C. Following hatching, chicks were maintained in the incubator with the temperature being reduced 2°C per day. Water and a commercial chick starter feed were freely accessible to the hatchlings.

Tissue Preparation. At various stages of incubation or post-hatching, livers and small intestines (the section immediately adjacent to the gizzard) were excised. Tissues used for Western blotting were weighed and homogenized in 3 volumes of homogenization buffer (1mM TrisCl, pH 7.5; 250 mM sucrose; 0.5 mM dithiothreitol; 20 mM leupeptin) using a Potter Elvehjem type homogenizer. An aliquot was removed for protein determination and a portion of the remainder was diluted in SDS sample buffer (5% SDS; 10% mercaptoethanol; 0.01% bromphenol blue and 5% glycerol) and boiled for three to five minutes.

Electron Microscopy. Tissues to be used for electron microscopy were fixed and embedded in Lowicryl K4M resin (14). Sections were cut and placed on Formvar coated grids. Immunostaining was accomplished by inverting the grids over a droplet of tris buffered saline (TBS; 20 mM TrisCl, pH 7.5; 500 mM NaCl) containing 1% gelatin for 10 min followed by three hours on a similar droplet containing 1:100 rabbit anti-rat SCP_2 anti-serum. The samples were briefly washed with TBS and inverted over a droplet of TBS containing 1% gelatin and 1:50 goat anti-rabbit IgG-10 nm colloidal gold conjugate for 3 additional h at room temperature. Sections were washed, lightly counterstained with uranyl acetate and examined in a Zeiss model 10B electron microscope.

Gel Electrophoresis and Western Blotting. Proteins (100 mg, as determined by the method of Bradford (15)) were electrophoresed on duplicate 15% polyacrylamide SDS gels. One gel was stained with Coomassie Brilliant Blue R250 while the duplicate was blotted onto 0.2 μm pore size nitrocellulose by the method of Burnette (16) using a Bio-Rad immunoblot chamber. The blot was washed in TBS containing 1% Bovine Serum Albumin (BSA) and 0.05% Tween-20 for 15 min before the direct addition of rabbit anti-rat SCP_2 antiserum at a final dilution of 1:2000. The blot was incubated overnight at 4°C followed by one h at room temperature and was washed with five changes of TBS-Tween 20 prior to incubation in TBS-Tween 20:1% BSA containing goat anti-rabbit IgG conjugated with horse radish peroxidase (1:2000; Bio-Rad). After a two h incubation at room temperature, blots were rinsed and developed using 4-chloro-1-naphthol and H_2O_2 as substrates.

RESULTS AND DISCUSSION

Mobilization of lipid from the yolk to the body of the developing chicken does not proceed during the first two weeks of embryogenesis (17,18,19,20). The last week of development, however, is a time of rapid lipid transport (21). The yolk serves as the sole source of cholesterol for the embryo (except in the brain) and this cholesterol undergoes esterification in the yolk sac membrane prior to mobilization (22), indicating active movement to the site of acyl-CoA cholesterol acyltransferase (ACAT) in these cells. Since SCP_2 is thought to be involved in this process (7) we recently examined the presence of SCP_2 in yolk sac membrane cells, as well as in other tissues active in lipid transport and metabolism: namely liver and intestine. While SCP_2 was present in yolk sac membrane, it was difficult to detect by Western blotting and its abundance did not appear to correlate with the rate of lipid transport through these cells. Liver and intestine, however, not only contained substantial quantities of SCP_2 but showed changes in both absolute amount and molecular mass as hatching approached. The purpose of this study was to extend our observations to chick liver and intestine immediately following hatching. This period is marked by the rapid depletion of cholesterol esters which accumulate in the liver the week before hatching and the start of digestive and absorptive processes in the intestine. The relative abundance and molecular mass of SCP_2 found in liver and small intestine of chick embryos and hatchlings are pictured in Figure 1. It is seen that SCP_2 is present in liver tissue before, during and after hatching (Fig. 1). A total shift in the molecular mass species present is not seen in liver, though multiple molecular mass species are present. The 12 kDa SCP_2 is elevated several fold after hatching when compared with prehatching or the day of hatching. It is evident that little 12 kDa SCP_2 is present in small intestine of 18 day embryonic chick. At the time of hatching, however, SCP_2 of the 64 kDa form is abundant.

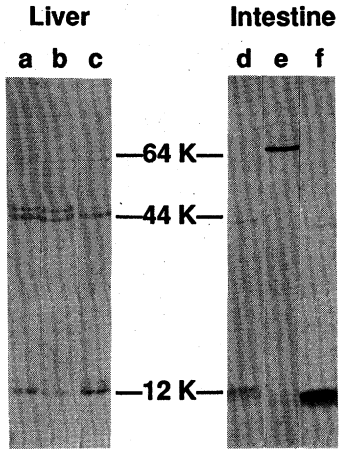

Figure 1. Western blot analysis of SCP_2 from chicken liver and intestine. Samples labeled a,b,and c are day 18 embryo, hatchling, and 2 day old chick liver, respectively (100 μg protein per lane). A substantial increase in 12 kDa immunoreactive material is seen after hatching. However, at all times, the major immunoreactive substance (a doublet) is 44 kDa. Intestinal samples are labeled d,e, and f which represent day 18 embryo, hatchling, and 2 day old chick, respectively. Little immunoreactive material is present in d (though what is present is at 12 kDa). e contains abundant immunoreactive material at 64 kDa, which in sample f is replaced by 12 kDa SCP_2.

Shortly after hatching this 64 kDa molecule disappears and is replaced with 12 kDa SCP_2. At this time, the amount of 12 kDa SCP_2 is several fold higher than that prior to hatching.

It is known that these three species also are present in mammalian tissue (11,12,13). In the rat, for example, SCP_2 with molecular masses of approximately 14, 36 and 58 kDa have been described. Each of these forms is found in tissue homogenates, and the 58 kDa form of the protein also is found in bile (14). While little is known of the functions of these different forms, their molecular and cell biology is becoming better understood. In the rat, at least three SCP_2 mRNA species are present (23,24). Currently, it is thought that each mRNA codes for a unique size of protein with the full sequence of the 14 kDa SCP_2 being found at the carboxyl terminus. This begs the question of whether these mRNAs arise as products of three distinct genes or if they are each processed from the RNA transcribed from a single gene. At present, it is thought that a single gene coding for SCP_2 is present and that each form of the mRNA is processed from a larger transcript derived from this single gene (24). What determines the tissue ratio of the different species is not known.

We have not yet demonstrated that three forms of mRNA are present in the chicken nor that each results in the synthesis of a distinct SCP_2 specie. If this is the case, however, our data strongly suggest that significant changes in the synthesis and processing of SCP_2 mRNA transcripts occur in the small intestine flanking the time of hatching These changes

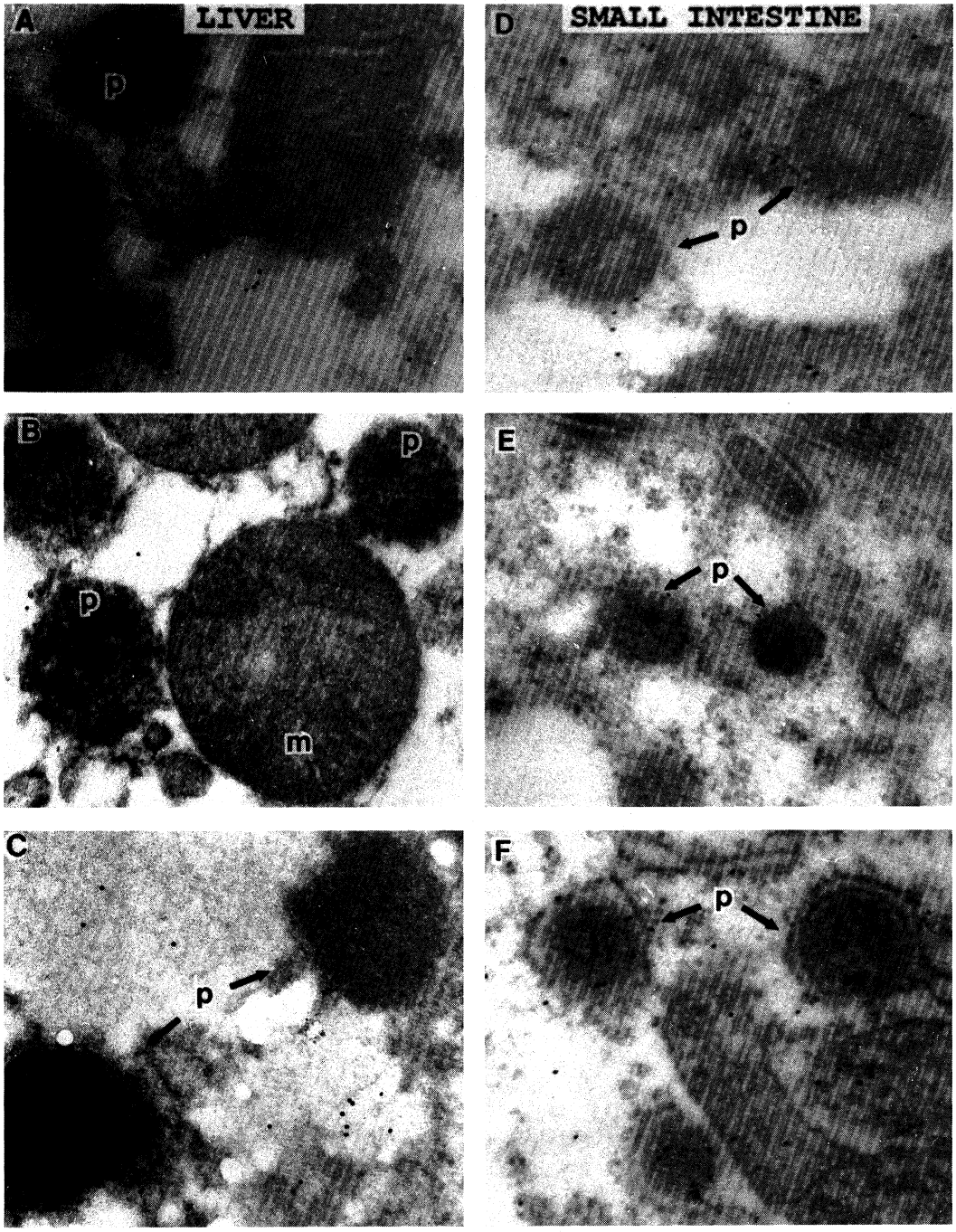

Figure 2. Immunogold labeling of SCP_2 in chick liver and intestine. Liver tissue obtained from 18 day embryos (A), hatchlings (B), and 2 day old chicks (C). At each stage, immunogold label is seen localized over peroxisomes (p). Mitochondria (m) also contain immunogold particles, but to a lesser degree. Samples of small intestine obtained at the same stages and are shown in the second column. Little staining is seen in the 18 day embryo sample (D). Peroxisomes are heavily stained in hatch day and 2 day old chick small intestine samples (E and F, respectively). Peroxisomes (p) are tightly encircled with what appears to be rough endoplasmic reticulum.

result first in increased accumulation of the higher molecular mass form of SCP_2 followed by a return to the lower mass form. In the liver, the only noticeable change is that more of the 12 kDa form is produced. The chicken small intestine, near the time of hatching, therefore represents an opportune system for examining the mechanisms regulating the different molecular forms of SCP_2.

In our earlier studies of the subcellular localization of SCP_2 in yolk sac membrane, liver and intestine of developing chick, we observed that the majority of immunostaining material was localized in peroxisomes (25). Figure 2 shows that liver SCP_2 is restricted mainly to peroxisomes before, during and after hatching. Additionally, SCP_2 immunostaining material can be found in liver mitochondria and, to a much lesser extent, on the endoplasmic reticulum. Thus the subcellular distribution of chick liver SCP_2 forms appears to remain constant throughout development and after hatching, and matches what has been observed in studies of mammalian SCP_2 localization which were carried out with rat liver (26,27). In this study, we also sought to determine if the shift in SCP_2 mass at the time of hatching might result in altered subcellular localization. Results shown in Figure 2, however, demonstrate that such alteration of distribution does not occur in intestinal tissue. Thus, no matter which molecular mass form is dominant, it appears to localize to the peroxisome. If the different molecular mass forms of chicken SCP_2 are like those of the rat, this is not surprising, since the peroxisome target signal resides at the extreme carboxyl terminus (15). In the rat, each molecular form contains the full SCP_2 sequence at the carboxyl terminus, thus having a peroxisome targeting sequence always available. These observations again raise the important but still unanswered question of SCP_2's real biological function.

ACKNOWLEDGMENT

We would like to thank Mr. Joseph Uknalis for his assistance in the preparation of the electron micrographs for this study.

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